Report to the Senate Armed Services Committee and the House of Representatives National Security Committee

on

Department of Defense Animal Care and Use Programs 1997

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List of Acronyms

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care International
AALAS	American Association of Laboratory Animal Science
ACLAM	American College of Laboratory Animal Medicine
APHIS	Animal and Plant Health Inspection Service
ASBREM	Armed Services Biomedical Research Evaluation and Management
AWA	Animal Welfare Act
AWIC	Animal Welfare Information Center
BRD	Biomedical Research Database
CNS	Central Nervous System
CRISP	Computer Retrieval Information on Scientific Projects
DDR&E	Director, Defense Research and Engineering
DoD	Department of Defense
DTIC	Defense Technical Information Center
FDA	Food and Drug Administration
FEDRIP	Federal Research in Progress
FY	Fiscal Year
IACUC	Institutional Animal Care and Use Committee
IG	Inspector General
ILAR	Institute of Laboratory Animal Research
IRAG	Interagency Regulatory Alternatives Group
JDL	Joint Directors of Laboratories
JTCG	Joint Technology Coordinating Groups
LAM	Laboratory Animal Medicine
MATRIS	Manpower and Training Research Information Services
NIH	National Institutes of Health
NMR	Nuclear Magnetic Resonance
NOS	Nitric Oxide Synthase
NRC	National Research Council
OPRR	Office for the Protection from Research Risks
OSD	Office of the Secretary of Defense
PCR	Polymerase Chain Reaction
PHS	Public Health Service
POC	Point of Contact (Primary Contact)
RDT&E	Research, Development, Test, and Evaluation
S&T	Science and Technology
STO	Science and Technology Objective
TAPSTEM	Training and Personnel Systems Science and Technology Evaluation and Management
USAMRMC	United States Army Medical Research and Materiel Command
USDA	United States Department of Agriculture
VEE	Venezuelan Equine Encephalitis
WRAIR	Walter Reed Army Institute of Research

SECTION V
DoD ANIMAL USE PROFILES

The information presented in this section provides profiles on the use of animals in various research categories, and the U.S. Department of Agriculture (USDA) pain categories of Department of Defense (DoD) animal-based research, testing and training programs for fiscal year (FY) 1997.

V.1 METHODS

Information was solicited and received from DoD agencies and military commands, organizations, and activities involved in animal care and use programs located both inside and outside of the United States. This included extramural contractors and grantees that performed animal-based research. For the purpose of this reporting requirement, an intramural program represents research performed at a DoD facility and funded by either DoD or non-DoD funds. An extramural program represents research performed by a contractor or grantee that is funded by the DoD.

V.1.1 Animal Use Profiles

The animal use profiles prepared for this report are consistent with the reporting information and data provided to the USDA using Animal and Plant Health Inspection Service (APHIS) Form 7023. In addition, this report contains comprehensive information on all other animals (e.g., mice, rats, birds) used that are not required in reports to the USDA.

For the purposes of this reporting requirement, an animal was defined as any whole nonhuman vertebrate, living or dead, excluding embryos, that was used for research, development, test, and evaluation (RDT&E), clinical investigations, diagnostic procedures, and/or instructional programs. Only live animals or whole dead animals, as defined, that were either on hand in the facility or acquired during FY97 and used are included. Animal organs, tissues, cells, blood, fluid components, and/or by-products purchased or acquired as such animal/biological components are not reported. This definition does not include animals used or intended for use as food for consumption by humans or animals, animals used for ceremonial purposes, or military working animals and their training programs.

A single animal was counted only once in determining the number of animals used during the fiscal year for a particular work unit or protocol. This does not refer to the number of times an individual animal was injected, manipulated, handled, or administered medication and/or experimental compounds within a given work unit, protocol, or program. Animals on hand during FY97, but not actually used during the fiscal year, are not included in this number.

V.1.2 Animal Use Categories

All DoD agencies and military commands, organizations, and activities involved in the performance and/or funding of animal care and use programs reported animal work by the category that best describes the general purpose of the animal use. If these categories did not describe the animal use within a particular work effort, the animal was placed under the Other category. The 8 general categories and 23 specific subcategories are listed in Table V-1. In-depth information on specific activities performed within a subcategory is presented in Appendix N. The medical research categories correspond to the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee's Joint Technology Coordinating Group Medical Research Areas. Non-medical categories consist of RDT&E programs performed outside the ASBREM Committee medical oversight. Clinical Investigations studies were performed under the auspices of the Assistant Secretary of Defense for Health Affairs and the military services medical departments through Major Force Program 8 funding. These studies were usually in support of graduate medical education training programs located at the major military medical centers.

Table V-1 Animal Use Categories

MEDICAL (M)
- M1: Military Dentistry
- M2: Infectious Diseases
- M3: Medical Chemical Defense
- M4: Medical Biological Defense
- M5: Human Systems Technology
- M6: Combat Casualty Care
- M7: Ionizing Radiation
- M8: Other Medical RDT&E

NON-MEDICAL (N)
- N1: Physical Protection
- N2: Physical Detection
- N3: Offensive Weapons Testing
- N4: Other Non-Medical RDT&E

CLINICAL INVESTIGATIONS (C)
- C1: Clinical Medicine
- C2: Clinical Surgery
- C3: Other Clinical Investigations

TRAINING/INSTRUCTIONAL (T)
- T1: Training, Education, and/or Instruction for Personnel
- T2: Other Training/Instruction

ADJUNCTS/ALTERNATIVES TO ANIMAL STUDIES (A)
- A1: Adjuncts to Animal Use Research
- A2: Alternatives to Animal Investigation
- A3: Other Alternatives/Adjuncts

CLASSIFIED SECRET OR ABOVE STUDIES (S): Classified secret or above studies on animals

ANIMAL BREEDING STOCK (B): Animals maintained for breeding

OTHER ANIMAL USE CATEGORIES (O): Other animal use purposes

V.1.3 USDA Pain Categories

The USDA requires that all institutions using any regulated animal for research, testing, training, or experimentation register with the USDA as a research facility and submit an annual report. This annual report presents the number of regulated animals used and the type of pain, if any, the animals were exposed to.

The USDA has developed three pain categories for its reporting requirement (TableV-2). All animals herein reported are assigned to one of the three USDA pain categories; this includes animals that are not regulated by the USDA. The USDA requires that any reporting facility that uses procedures producing unalleviated pain or distress file an explanation of the procedures with its annual APHIS report.

The animals reported in Column C of the USDA report are those used in procedures that are not painful. Procedures performed on these animals are those that are usually conducted on humans without anesthesia or analgesia. Examples include most blood-sampling techniques (excluding intracardiac and periorbital blood sampling), injections, and tattooing.

The animals reported in Column D of the USDA report are those that experience pain in which appropriate anesthetic, analgesic, or tranquilizing drugs were used. Examples include anesthesia for surgical procedures or catheter placement, and analgesia during recovery from surgery.

The animals reported in Column E of the USDA report are those that experience more than slight or momentary pain or distress that cannot be alleviated by drugs. Examples of procedures where drugs were not used because they would have adversely affected the procedures, results or interpretation of the research, or tests include some infectious disease studies and some toxicology studies.

All procedures that involve animals in Columns D or E are extensively reviewed during the protocol approval process. A veterinarian with experience and/or training in laboratory animal medicine must review all procedures that could cause pain and distress in animals prior to formal protocol review. In addition, the primary investigator must write a justification for all procedures for animals in

Table V-2 USDA Pain Categories (USDA APHIS Form 7023)

USDA COLUMN C
Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.

USDA COLUMN D
Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.

USDA COLUMN E
Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.

Columns D and E. The DoD standard protocol states, "Procedures causing more than transient or slight pain that are unalleviated must be justified on a scientific basis in writing by the primary investigator. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized." Moreover, the primary investigator must sign an assurance statement that alternative procedures are not available, and the Institutional Animal Care and Use Committee must review and approve all procedures before the study begins.

V.2 Results/Discussion

V.2.1 General Results

There was a total of 316,048 animals used in FY97 which is a 1% decrease from FY96 and a 43% decrease from FY93 (Figure V-1). The Animal Welfare Act of 1985 defines animals as "any live or dead dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warm-blooded animal, as the Secretary may determine..." Therefore, only 7% (22,014) of the animals used by the DoD in FY97 are considered USDA reportable species.

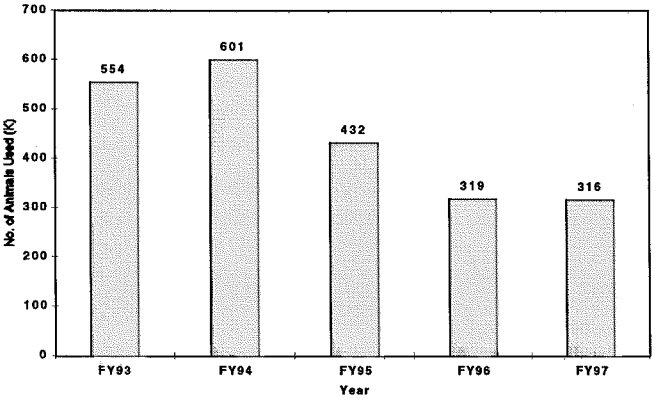


***Figure V-1** DoD Animal Use by Year*

In FY97, 149,187 animals were used in intramural research programs and 166,861 were used in extramural grants or contracts (Figure V-2). Intramural animal use decreased by 17%, (30,968) in FY97 compared with FY96 use and decreased by 44% (118,904) compared with FY94 use. While the number of animals used in extramural research was 20% (28,216) greater in FY97 than the number in FY96, it was 50% (165,731) lower than the number used in FY94. Extramural programs by their very nature have large

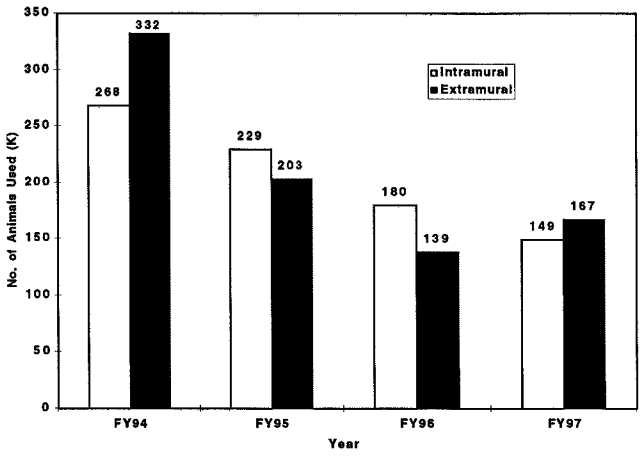


***Figure V-2** Intramural/Extramural Animal Use by Year*

fluctuations in the number of animals used from year to year. Each year a different number of contracts are granted to perform extramural research. Many of these do not use animals at all; others only use animals during a portion of the proposed project (e.g., third year of project); and others use animals throughout the entire project. In addition, the level of funding for extramural programs varies from year to year thereby changing the total number of extramural projects. Some extramural research programs are congressionally mandated such as Breast Cancer, Neurofibromatosis, Osteoporosis Research Programs; their funding is dependent on yearly congressional appropriations. Therefore, changes in the number of animals used by the DoD extramural research programs can fluctuate significantly from year to year. The intramural programs have less variation in their use of animals because they have a continuous mission and ongoing research in specific areas. Consequently, any decrease in the number of animals used is most likely a result of the use of alternatives to animal use, a decrease in the number of research projects, or a decrease in intramural funding.

V.2.2 Animal Use by Service

Information concerning total DoD use of animals by each service is presented in Figure V-3. Figures V-4 and V-5 show the intramural and extramural animal use by service, respectively.

In FY97, the Army used 72% of the total number of animals used by the DoD, 65% of the intramural animals, and 78% of extramural animals. There was

TOTAL = 316,048

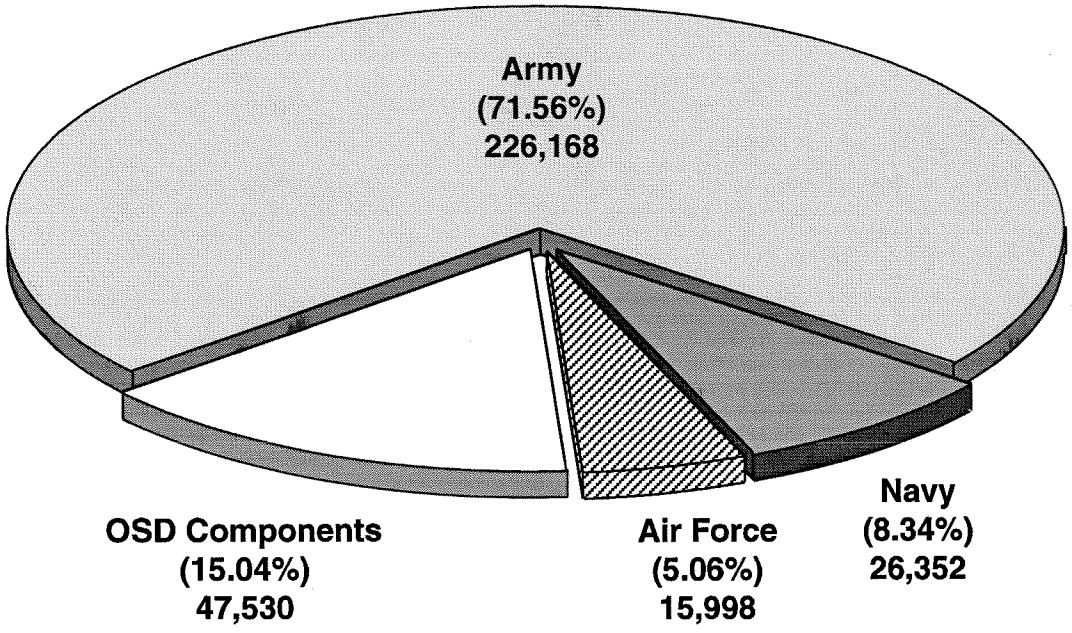


Figure V-3 *DoD Intramural and Extramural Animal Use by Service for FY97*

TOTAL = 149,187

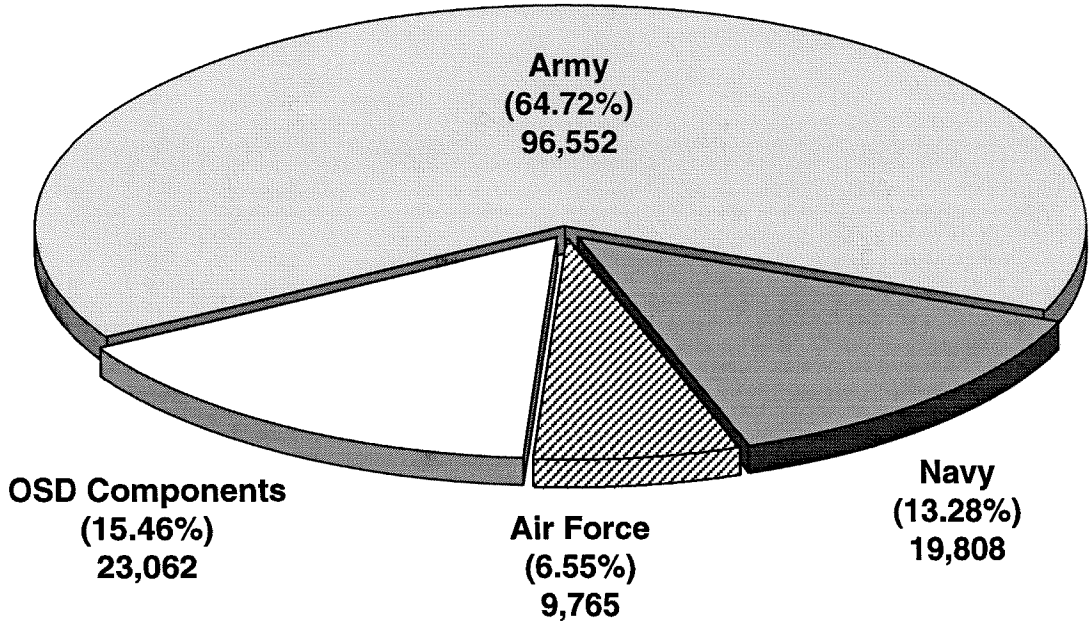


Figure V-4 *DoD Intramural Animal Use by Service for FY97*

Percentages may not add up to 100% due to rounding of calculations

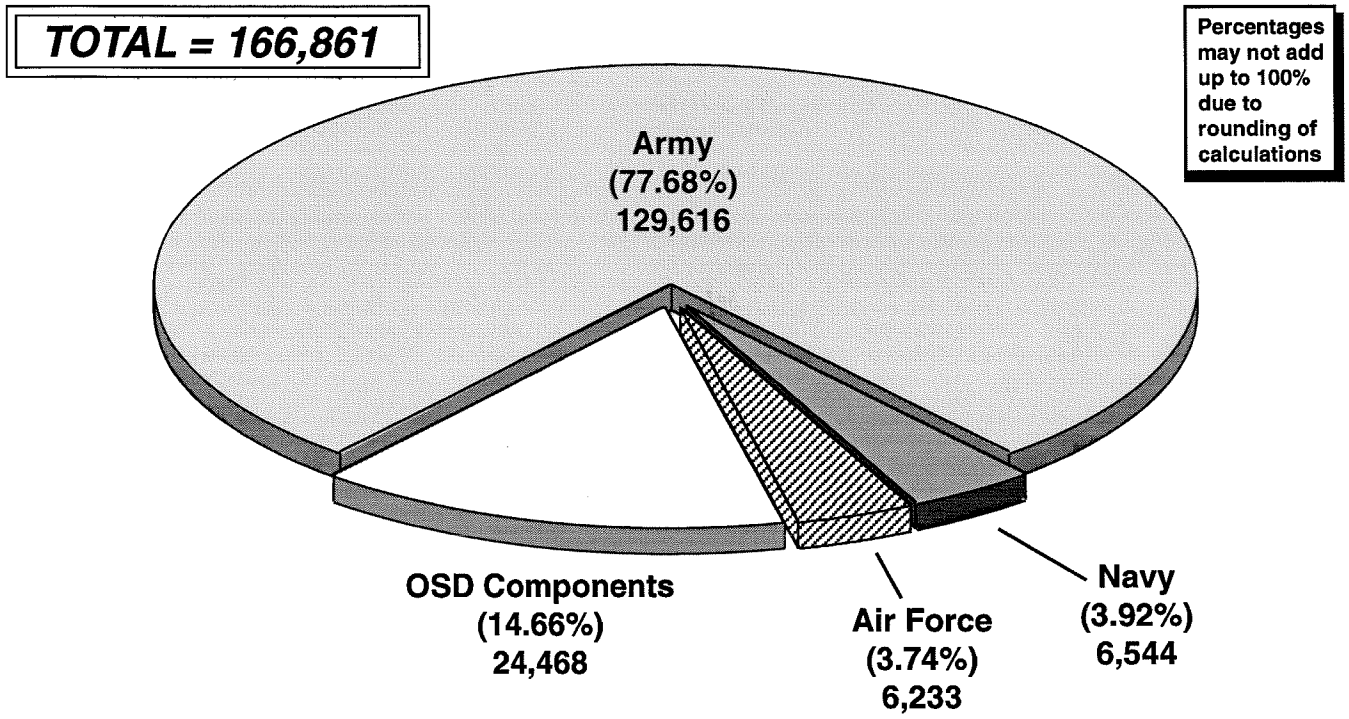


Figure V-5 DoD Extramural Animal Use by Service for FY97

a 9% decrease in the Army's intramural animal use and a 4% increase in extramural animal use since FY96. The Army has an ongoing responsibility to manage the congressionally mandated Breast Cancer, Neurofibromatosis, Osteoporosis, and Defense Women's Health Research Programs. These programs used the majority of the Army's extramural research animals (48,908). The U.S. Army Medical Research and Materiel Command is the congressionally mandated Lead Agency for infectious disease and combat dentistry research and the DoD Executive Agent for medical chemical and biological defense and nutrition studies. The number of animals the Army used in research on infectious diseases and chemical and biological defense was 61,445 and 74,597, respectively.

The Navy used 8% of the total number of animals used by the DoD, 13% of the intramural animals, and 4% of extramural animals. Comparing animal use in FY97 with use in FY96, there was a 29% (10,516) reduction in the total number of animals used by the Navy. Animal use in the Navy's intramural research projects decreased (10,915) while the extramural projects demonstrated a slight increase (399) in animal use. The majority of animals used by the Navy were in infectious disease research (17,014).

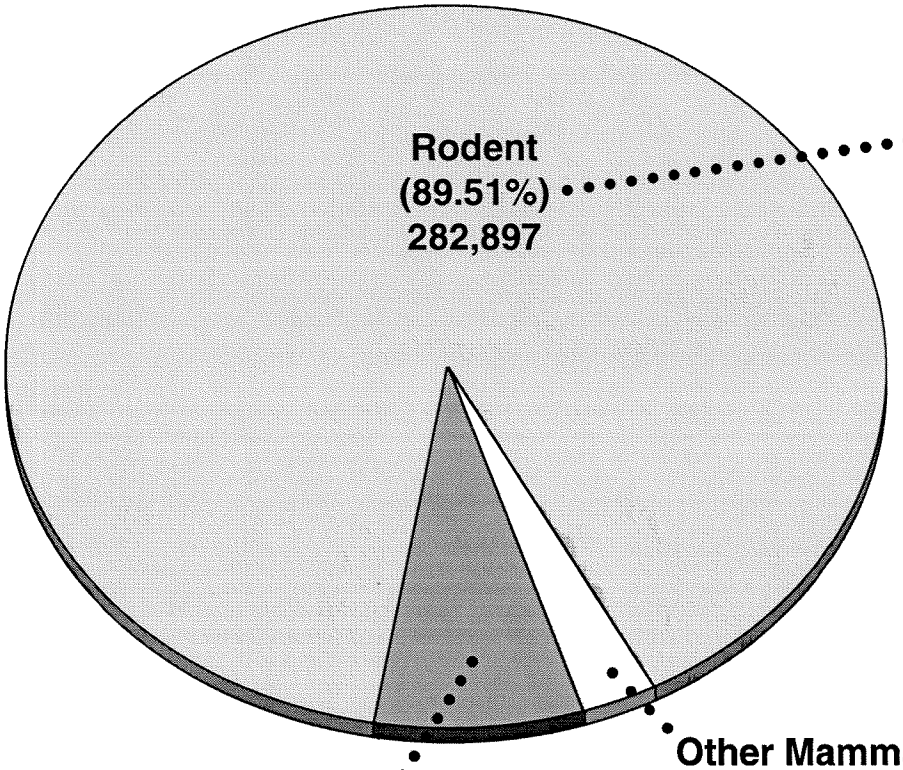
The Air Force used 5% of the total number of animals used by the DoD, 7% of the intramural animals, and 4% of the extramural animals. The Air Force had a slight (2%) increase in intramural animal use and a 16% decrease in extramural animal use resulting in a 6% (995) overall decrease in the number of animals used in research in FY97 compared with FY96. The Air Force used the majority of animals in non-medical research projects (10,789).

The Office of the Secretary of Defense (OSD) components are the Uniformed Services University of the Health Sciences, Defense Advanced Research Projects Agency, Armed Forces Radiobiology Research Institute, and Armed Forces Institute of Pathology. OSD components used 15% of the DoD total animals used, 15% of the total intramural animals, and 15% of total extramural animals. There was a 42% (14,122) increase in the use of animals for the OSD components in FY97 compared with FY96. Over 90% of this increase was in OSD components extramural programs. The OSD components used the majority (96%) of their animals in clinical investigations and medical research.

V.2.3 Animal Use by Species

DoD animal use by species is presented in Figure V-6. Figures V-7 and V-8 represent the intramural and extramural animal use by species for FY97.

TOTAL = 316,048



Rodent (89.51%) 282,897

Chinchilla	193	(0.06%)
Chipmunk	18	(0.01%)
Degu	34	(0.01%)
Gerbil	77	(0.02%)
Guinea Pig	7,602	(2.41%)
Hamster	3,960	(1.25%)
Jird	24	(0.01%)
Mouse	227,303	(71.92%)
Rat	42,272	(13.38%)
Shrew	8	(<0.01%)
Squirrel	36	(0.01%)
Vole	1,370	(0.43%)

Other Mammals (2.75%) 8,692

Bat	26	(0.01%)
Burro	2	(<0.01%)
Cat	57	(0.02%)
Cow/Bull	8	(<0.01%)
Dog	342	(0.11%)
Ferret	211	(0.07%)
Goat	839	(0.27%)
Horse	4	(<0.01%)
Marine Mammal	303	(0.10%)
Nonhuman Primate	1,527	(0.48%)
Opossum	6	(<0.01%)
Pig/Swine	2,274	(0.72%)
Rabbit	2,709	(0.86%)
Raccoon	14	(<0.01%)
Sheep	370	(0.12%)

Marine Mammals include: Beluga Whale (5), Bottlenose Dolphin (51), California Sea Lion (4), Elephant Seal (20), False Killer Whale (2), Gray Whale (1), Killer Whale (1), Marine Mollusc (168), Northern Elephant Seal (46), Pacific Harbor Seal (1), Pacific White-side Dolphin (3), Risso's Dolphin (1).

Nonhuman Primates include: Aotus Monkey (73), Baboon (24), Monkey (812), Rhesus Monkey (258), Other Nonhuman Primate (360).

Non-Mammal (7.74%) 24,459

Amphibian	2,517	(0.80%)
Avian	847	(0.27%)
Fish	20,959	(6.63%)
Reptile	136	(0.04%)

Amphibians include: African Clawed Frog (163), Frog (497), Salamander (1,204), Tadpole (212), Toad (441).

Avian include: Chicken (777), Crane (16), Goose (18), Pigeon (36).

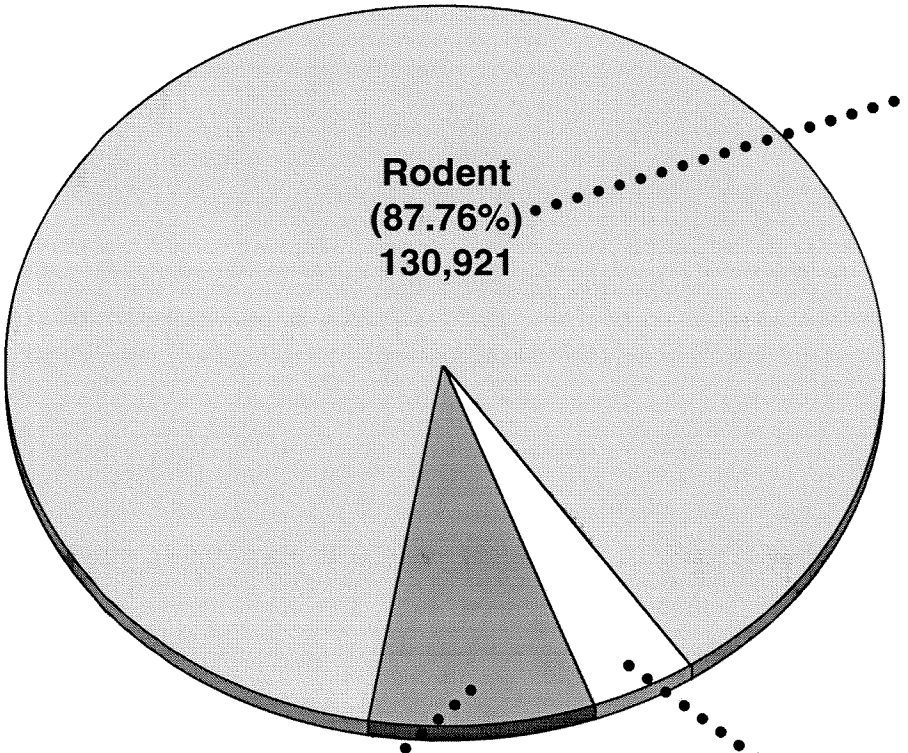
Fish include: Bluegill Sunfish (2,850), Boxfish (7), Eel (27), Fathead Minnow (1,680), Japanese Medaka (10,400), Kniff-fish (9), Milkfish (7), Rainbow Trout (1,000), Triggerfish (27), Zebra Fish (2,000), Other Fish (2,952).

Reptiles include: Iguana (42), Sea Turtle (2), Snake (92).

Percentages may not add up to 100% due to rounding of calculations

Figure V-6 *DoD Intramural and Extramural Animal Use by Species for FY97*

TOTAL = 149,187



Chinchilla	145	(0.10%)
Chipmunk	18	(0.01%)
Gerbil	42	(0.03%)
Guinea Pig	4,700	(3.15%)
Hamster	2,448	(1.64%)
Jird	24	(0.02%)
Mouse	102,712	(68.85%)
Rat	20,752	(13.91%)
Shrew	8	(0.01%)
Squirrel	7	(<0.01%)
Vole	65	(0.04%)

Amphibian	1,652	(1.11%)
Avian	213	(0.14%)
Fish	10,710	(7.18%)
Reptile	55	(0.04%)

Amphibians include: African Clawed Frog (35), Frog (200), Salamander (1,204), Tadpole (212), Toad (1).

Avian include: Chicken (143), Crane (16), Goose (18), Pigeon (36).

Fish include: Bluegill Sunfish (2,850), Japanese Medaka (7,000), Other Fish (860).

Reptiles include: Iguana (12), Snake (43).

Burro	2	(<0.01%)
Cat	35	(0.02%)
Cow/Bull	3	(<0.01%)
Dog	157	(0.14%)
Ferret	142	(0.11%)
Goat	818	(0.55%)
Horse	4	(<0.01%)
Marine Mammal	204	(0.14%)
Nonhuman Primate	954	(0.64%)
Opossum	6	(<0.01%)
Pig/Swine	1,536	(1.03%)
Rabbit	1,510	(1.01%)
Raccoon	14	(0.01%)
Sheep	187	(0.13%)

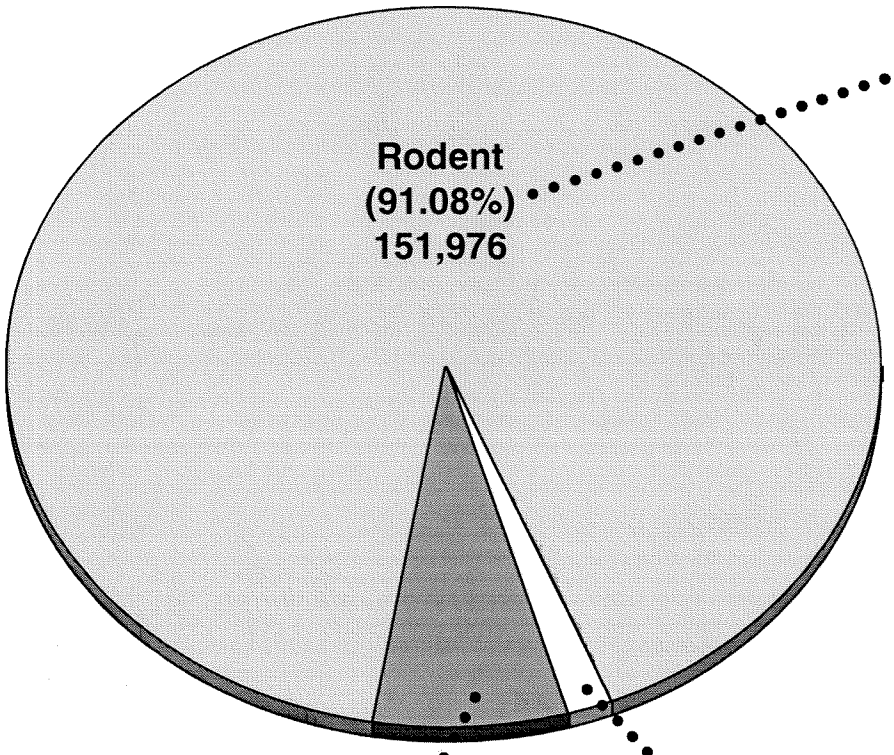
Marine Mammals include: Beluga Whale (3), Bottlenose Dolphin (31), False Killer Whale (1), Marine Mollusc (168), Risso's Dolphin (1).

Nonhuman Primates include: Aotus Monkey (73), Rhesus Monkey (246), Other Monkey (287), Other Nonhuman Primates (348).

Percentages may not add up to 100% due to rounding of calculations

***Figure V-7** DoD Intramural Animal Use by Species for FY97*

TOTAL = 166,861



Chinchilla	48	(0.03%)
Degu	34	(0.02%)
Gerbil	35	(0.02%)
Guinea Pig	2,902	(1.74%)
Hamster	1,512	(0.91%)
Mouse	124,591	(74.67%)
Rat	21,520	(12.90%)
Squirrel	29	(0.02%)
Vole	1,305	(0.78%)

Non-Mammal
(7.09%)
11,829

Amphibian	865	(0.52%)
Avian	634	(0.38%)
Fish	10,249	(6.14%)
Reptile	81	(0.05%)

Amphibians include: African Clawed Frog (128), Frog (297), Toad (440).

Avian include: Chicken (634).

Fish include: Boxfish (7), Eel (27), Fathead Minnow (1,680), Japanese Medaka (3,400), Kniff Fish (9), Milkfish (7), Rainbow Trout (1,000), Triggerfish (27), Zebra Fish (2,000), Other Fish (2,09).

Reptiles include: Iguana (30), Sea Turtle (2), Snake (49).

Other Mammals
(1.83%)
3,056

Bat	26	(0.02%)
Cat	22	(0.01%)
Cow	5	(<0.01%)
Dog	136	(0.08%)
Ferret	54	(0.03%)
Goat	21	(0.01%)
Marine Mammal	99	(0.06%)
Nonhuman Primate	573	(0.34%)
Pig/Swine	738	(0.44%)
Rabbit	1,199	(0.72%)
Sheep	183	(0.11%)

Marine Mammals include: Beluga Whale (2), Bottlenose Dolphin (20), California Sea Lion (4), Elephant Seal (20), False Killer Whale (1), Gray Whale (1), Killer Whale (1), Northern Elephant Seal (46), Pacific Harbor Seal (1), Pacific White-side Dolphin (3).

Nonhuman Primates include: Baboon (24), Monkey (525), Rhesus Monkey (12), Other Nonhuman Primate (12).

Percentages may not add up to 100% due to rounding of calculations

Figure V-8 DoD Extramural Animal Use by Species for FY97

The majority (97%) of animals used by the DoD, both intramurally and extramurally, were rodents, birds, amphibians, reptiles, and fish.

The overall number of nonhuman primates, dogs, and cats continued to decrease in FY97 (Figure V-9). Compared with FY96, in FY97 there was a decrease in the use of nonhuman primates (284) and cats (21) and a slight increase in the use of dogs (65). Dogs were primarily used in medical and clinical research (71%).

Since FY94, there has been a 31% (682) decrease in the use of nonhuman primates and a 64% (704) decrease in the use of companion animals for research in the Department of Defense. This illustrates the Department's continuing commitment to reducing the use of specific species in research.

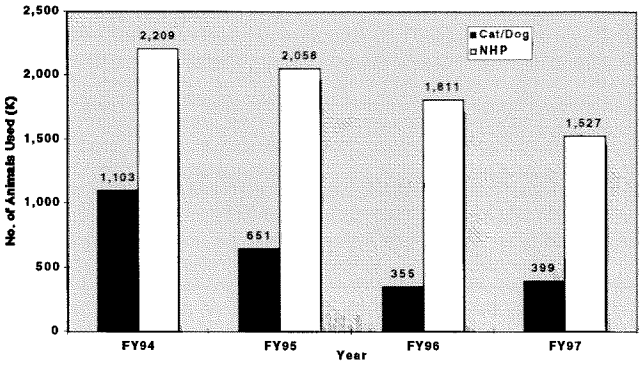
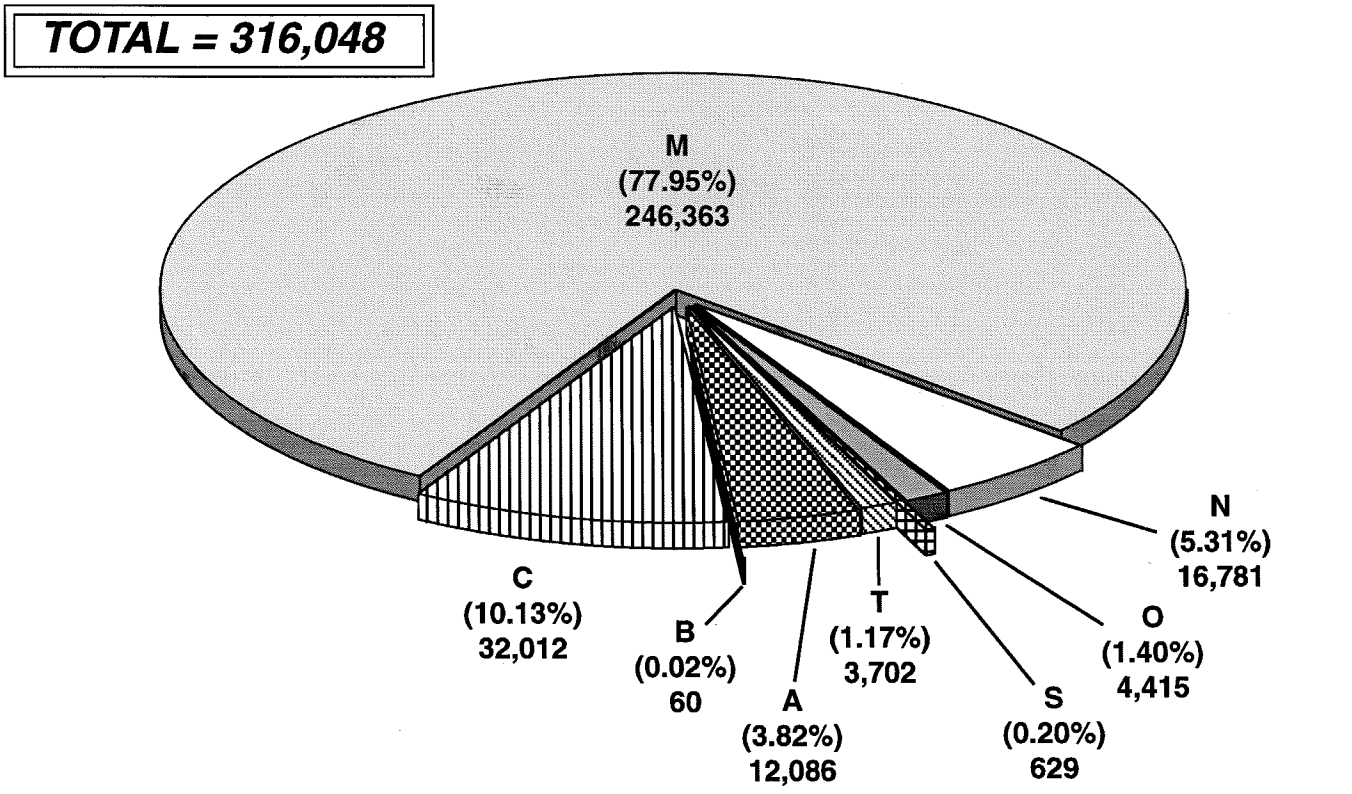


Figure V-9 *Use of Nonhuman Primates, Dogs, and Cats by Year*

V.2.4 Animal Use by Category

Total animal use in the DoD by category is presented in Figure V-10, with the intramural and extramural breakouts in Figures V-11 and V-12, respectively.



A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations

Figure V-10 *DoD Intramural and Extramural Animal Use by Category for FY97*

TOTAL = 149,187

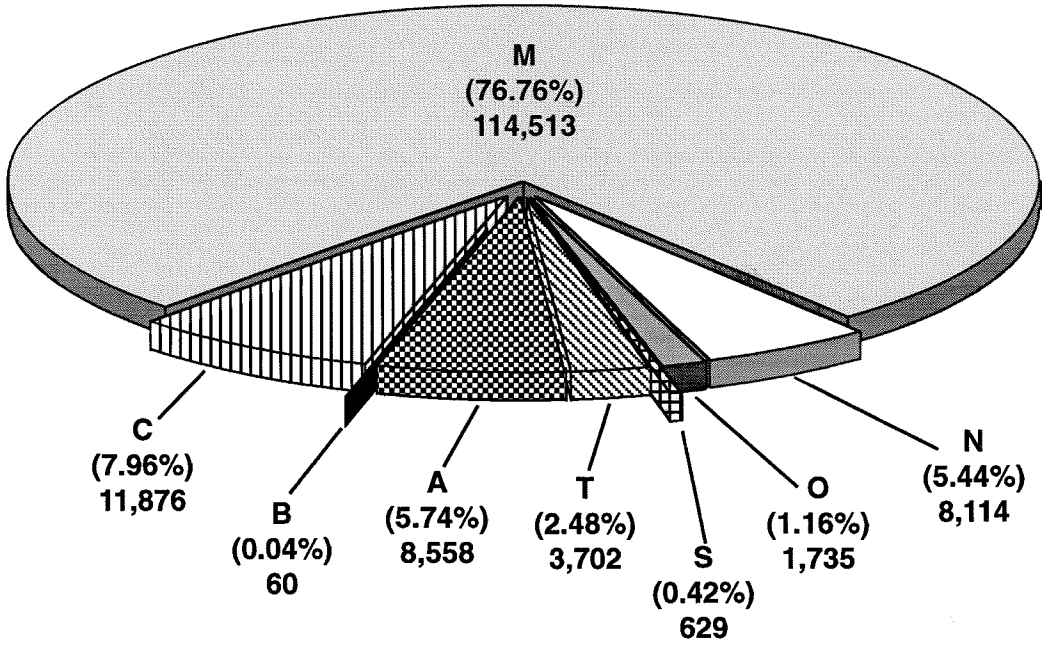


Figure V-11 *DoD Intramural Animal Use by Category for FY97*

TOTAL = 166,861

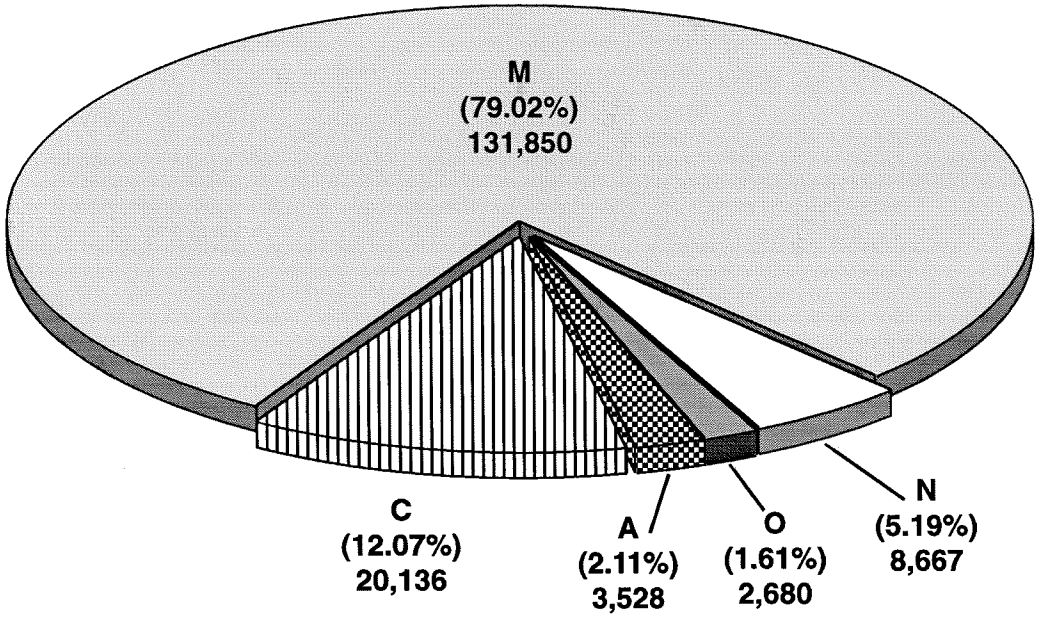


Figure V-12 *DoD Extramural Animal Use by Category for FY97*

A: Adjuncts/Alternatives to Animal Studies, B: Animal Breeding Stock, C: Clinical Investigations, M: Medical RDT&E, N: Non-Medical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations

The DoD has a critical and challenging mission: to discover, design, and develop military medical countermeasures against threats to the health and survivability of military personnel. In order to meet this mission, 78% of the animals used by the DoD in FY97 were in medical research. Thirty-five percent (87,043) of animals used in medical research were in the area of infectious diseases (M2) and were primarily rodents (99%) (Appendix O). The primary thrust of this research is the development of preventive measures against infectious disease through discovery, design, and development of prophylactic, therapeutic, and treatment drugs for relevant diseases. The chemical defense research program (M3) used 17% (40,966) and the biological defense research program (M4) used 14% (33,631) of the medical research animals. Medical biological defense develops, demonstrates, and fields new vaccines, drugs, and diagnostic kits for the prevention, treatment, and diagnosis of biological warfare agents. This research program protects the armed forces from the consequences of exposure to biological warfare agents and enhances their survivability. M8 (Other Medical Research) accounted for 24% of the total medical research category (Figure V-13). The congressionally directed research programs in the areas of breast cancer, Defense women's health, neurofibromatosis and osteoporosis used 48,908 animals. These programs accounted for 83% of M8 animals (Table V- 3), 20% of the animals used in medical research, and 16% of the total DoD animals used. These types of research programs can cause fluctuations in the total number of animals used from year to year depending on congressional funding levels and direction. Other areas of research within M8 are shown in Table V-3.

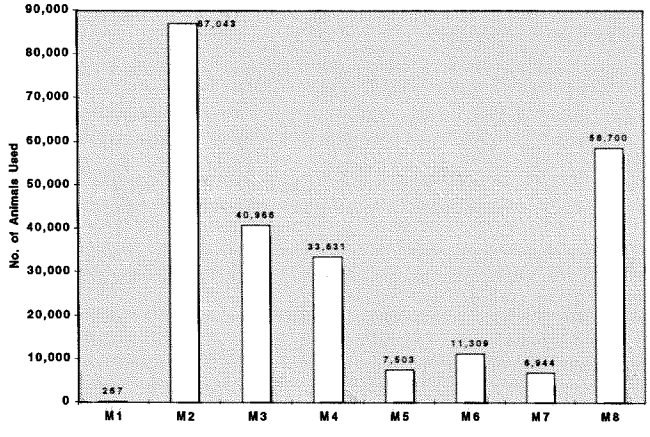


***Figure V-13** Animal Use by Medical Research Category*

Research Category (M8)	No. of Animals Used	Percentage of M8
Breast Cancer	45,077	76.79%
Diode Lasers	42	0.07%
Defense Women's Health Research Program	1,466	2.50%
Gulf War Illnesses	1,130	1.93%
Medical Free Electron Laser	928	1.58%
Neurobehavioral	288	0.49%
Neurofibromatosis	1,912	3.26%
Occupational Medicine	264	0.45%
Osteoporosis	453	0.77%
Pathophysiology	1,406	2.40%
Toxicology	2,339	3.98%
Vectorborne Disease Research and Training	6	0.01%
Zoonosis	3,389	5.77%
Total M8 Research	**58,700**	**100.00%**

***Table V-3** M8 (Other) Medical Research Category*

Clinical research accounted for 10% (32,012) of the animals used by the DoD in FY97. Studies in this category address clinical medicine and surgical problems for the treatment of both diseases and combat casualties. Ninety-two percent of the animals used in clinical research were used in clinical medicine studies. While many of these conditions are unique to the military, several are not. Specific types of clinical studies are listed in Appendix N.

One percent of the animals used by the DoD in FY97 were in the training, education, and instruction of personnel. Training and instruction are basically for animal technicians and medical personnel (Appendix N). There was a 45% decrease (2,978) in animals used in this category in FY97 compared with FY96. The reduction in the use of animals for training purposes reflects the DoD's commitment to using non-animal training techniques such as computer simulations and sharing of training animals whenever possible. Breeding stock, classified studies, and other studies accounted for less than 1% of the DoD's total animal use in FY97.

Non-medical RDT&E animal use decreased by 59% (24,164) in FY97 compared with FY96 and accounted for only 5% of the total animal use in FY97. Research in the area of alternatives to the use of animals was 4% of the total animal use for FY97. Research in this category illustrates the Department's continuing initiatives to promote research to develop alternatives to reduce, replace, and refine the use of animals in DoD research.

No animals were used for offensive weapons testing during FY97.

V.2.5 Animal Use by USDA Pain Category

Total animal use in the DoD by USDA pain category is presented in Figure V-14, with the intramural and extramural breakouts in Figures V-15 and V-16, respectively.

Most research (~80%) in the DoD was not painful to the animals involved. In the majority of the cases (52%), the animals were not exposed to or involved in any painful procedures. In 28% of the cases, animals were given anesthesia or pain-relieving drugs during procedures that could have involved some pain or distress to the animals. In 20% of the animals used, anesthetics or analgesics were not used because they would have interfered with the validity of the results of experiments. Most (98%) of the animals used in painful experiments (where reducing the pain or distress would have interfered with the validity of the results) were rodents. Less than 1% of the animals in USDA Pain Category E were other mammals and less than 1% were non-mammals.

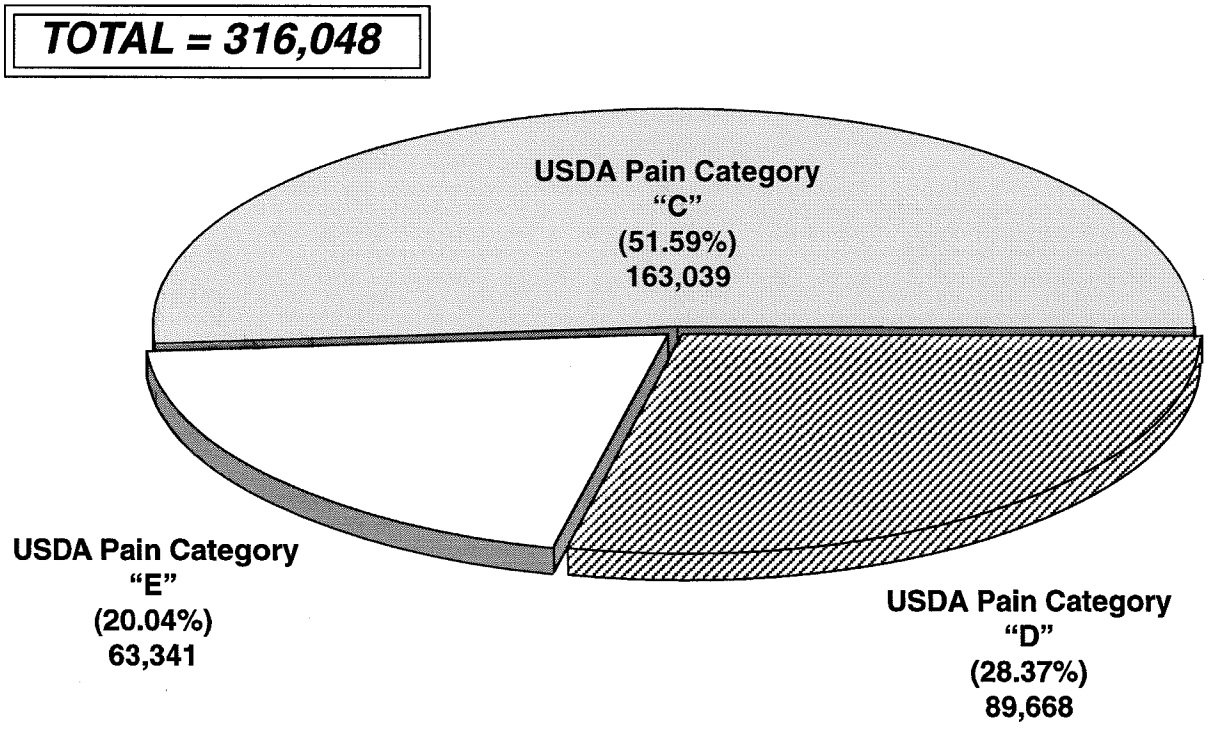


Figure V-14 *DoD Intramural and Extramural Animal Use by USDA Pain Category for FY97*

TOTAL = 149,187

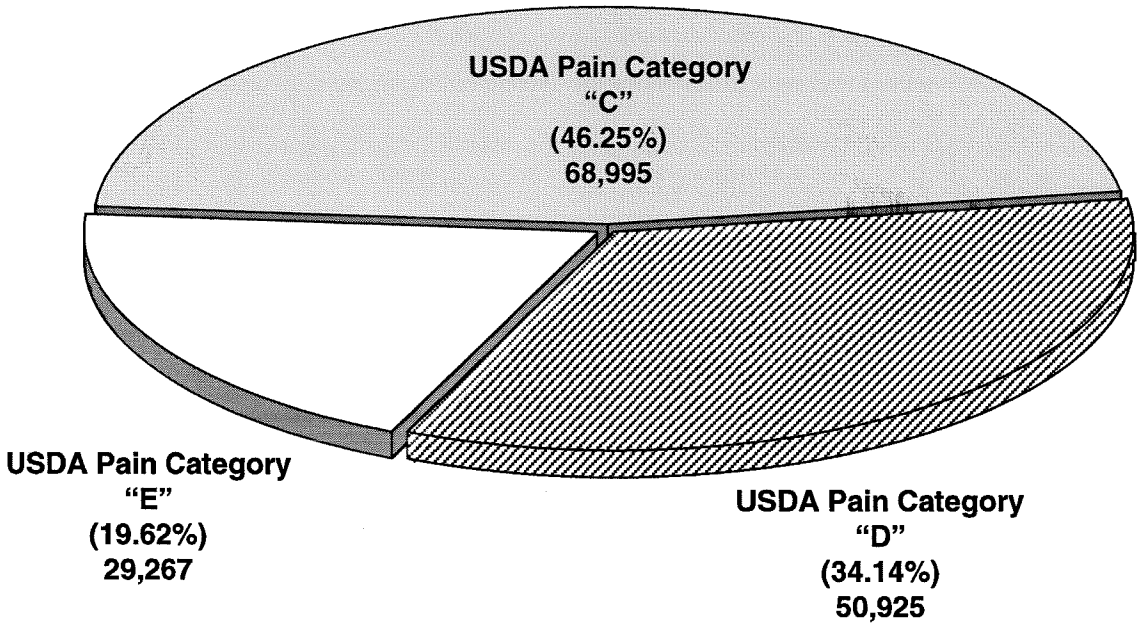


Figure V-15 DoD Intramural Animal Use by USDA Pain Category for FY97

Percentages may not add up to 100% due to rounding of calculations

TOTAL = 166,861

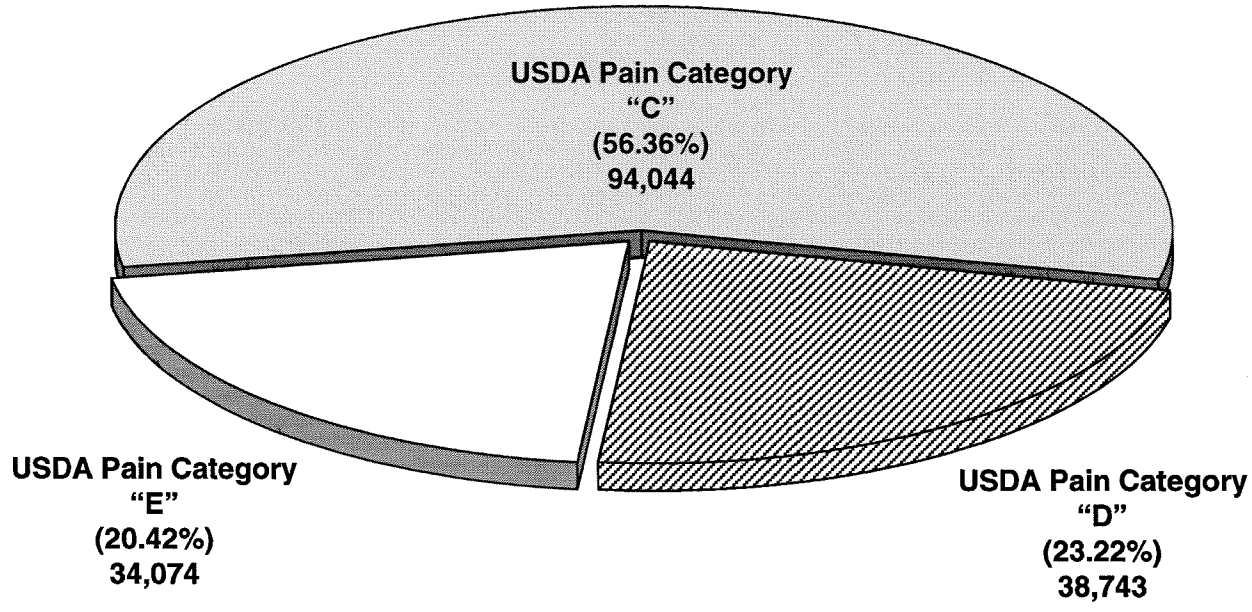


Figure V-16 DoD Extramural Animal Use by USDA Pain Category for FY97

Percentages may not add up to 100% due to rounding of calculations

Ninety-four percent of the animals reported in USDA Pain Category E were used in medical studies; of this, 87% of the animals were used in research on infectious disease and chemical and biological defense. Infectious disease and chemical and biological defense research usually falls into USDA Pain Category E because the animals have to be exposed to chemical or biological agents or other infections which may result in some type of distress. There were no animals subjected to unalleviated pain during training studies.

The DoD clearly has a most diverse, unique, and demanding R&D mission. The modern battlefield is a hostile and dangerous environment with extraordinary potential for exposure to lethal or debilitating conventional weapons, exotic endemic diseases, biological and chemical agents, nuclear blast and radiation, directed energy sources, and complex and dangerous equipment. In addition, a host of adverse environmental conditions, such as cold, heat, high and low pressure, and G-forces are threats to service men and women. The DoD must provide acceptable protection against these threats and many others. The animals reported in USDA Category E were used in research designed to find ways to protect service men and women from the threats they encounter daily. Note that in most of these studies the distress level is minor such as in heat stress or gastrointestinal distress after being exposed to G-forces. This critical research is often reliant upon animal models for vaccine and efficacious countermeasure development. Research of this kind is not commonly done elsewhere in the government, academic, or private sectors and therefore is the sole purview of the DoD. Also, a large portion of these studies are driven by federal requirements, particularly those of the Food and Drug Administration.

SECTION VI

DoD Initiatives to Promote Alternative Methods that Replace, Reduce and Refine the Use of Animals

Alternatives, as articulated in *The Principles of Humane Experimental Technique* (Russell and Burch, 1959), are defined as methods that **R**eplace, **R**educe and **R**efine the use of animals. In addition to these *Three Rs*, the Department of Defense (DoD) advocates a fourth *R*, "**R**esponsibility," for implementing these alternative methods.

Replacement

The replacement alternative addresses supplanting animal use with non-living systems, analytical assays, cell-culture systems, and with animals that are lower on the phylogenetic scale. Additionally, human subjects are used when experimental drugs and other procedures progress to human trials. Such trials are conducted in accordance with Title 32, U.S. Code of Federal Regulations, Section 219, "Protection of Human Subjects in DoD-Sponsored Research."

Reduction

Decreasing the number of animals used through the use of statistical or innovative design strategies, while preserving the scientific integrity of the biological model, is a major emphasis of the reduction alternative to animal use.

Refinement

The refinement alternative for animal use addresses the need to ensure that the maximum humane use of each animal is obtained through proper protocol design and efficient utilization of animals, or through the modification of the experimental design to reduce the ethical cost associated with the study.

Responsibility

The DoD has taken responsibility for implementing animal use alternatives. This commitment illustrates the DoD's initiative toward utilization and development of alternatives to animal use.

Department policy with regard to animal alternatives is promulgated in DoD Directive 3216.1 which directs that "it is DoD policy that... alternatives to animal species should be used if they produce scientifically satisfactory results...." This policy is implemented in the Joint Service Regulation on the Use of Animals in DoD Programs, which delegates responsibility to the local commander for utilization of alternatives to animals.

To illustrate the Department's initiatives to promote these *Four Rs*, a description of such initiatives within DoD's research laboratories and medical treatment centers is provided. The following list is not all inclusive, as the number of specific examples of implementing alternative methods that can be documented for DoD's research projects is large. Rather, it illustrates the scope, diversity, and spirit of DoD's *Four Rs* initiatives. This section will demonstrate a broad-based movement toward the use of biotechnology and other innovative adjuncts to replace and reduce animal use as well as refinement in methods used in essential animal studies.

VI.1 DoD Development of Animal Use Alternatives

A review of the FY97 DoD research reveals that 13 DoD facilities were actively involved in the development of alternatives to animal use, which is a 40% increase from FY96. These developments occur through both research specifically designed to produce alternatives and by research to improve experimental techniques. Whenever possible, DoD investigators attempt to develop state-of-the-art, scientifically relevant and reliable experimental procedures that can be performed without the use of animals. In addition, in cases where the animal models cannot be completely replaced, investigators work diligently to develop refinement techniques to reduce any stress placed on the animal during both experimental procedures and daily living. Examples of alternatives developed or in

development by DoD investigators reported in 1997 are listed below. This is only a sample of the alternatives developed or being developed this year.

Replacement:

- Rabbits replace nonhuman primates (NHPs) in the development of an *in vitro* correlate for anthrax.
- The South African clawed frog (*Xenopus laevis*) embryo replaces laboratory mammals commonly used in teratogenesis assays.
- Use of cell culture assays for determining the neutralization titers of mouse sera in Alphavirus vaccine research replaces the requirement for additional animals.
- Development of polymerase chain reaction techniques to detect *Coxiella burnetii* in clinical specimens will eliminate the need to detect the microorganism using experimental animals.
- The Japanese medaka fish used as an immune response biomarker should replace laboratory animals commonly used in toxicology research.
- Realistic biophysical models computationally simulate the damage processes induced by lasers as accurately as possible.
- Using the zebrafish as a model organism will replace higher order mammalian species for genetic analysis of vertebrate circadian rhythmicity.
- Artificial human skin and human whole blood can be used to delineate mechanisms and sites of action for stimulants and potential vesicant antagonists.
- Rabbit models will replace the use of dogs or NHPs in the study of compartment pressures in ischemia reperfusion.
- The free-ranging honeybee (*Apis mellifera*) is being used in place of animal sentinels to monitor contaminated environments.
- A technique was developed to examine individual neurons in culture, eliminating the need for intracranial surgery.
- Anatomical computer models of experimental animals and computer codes calculate where and how much microwave energy is absorbed in experimental animals when exposed to radiofrequency fields.
- An artificial eye with lenses that mimics the focusing characteristics of the eye can be used to expose this eye to various thresholds of laser exposures.

Reduction:

- The guinea pig model of anthrax vaccine adsorbed against strains of *Bacillus anthracis* from different, worldwide geographical areas will reduce the number of rabbits and NHPs that would be used to investigate *Bacillus anthracis* strain difference.
- A computer-based dosimetry model on the effects of microwave radiation on cognitive performance will reduce the numbers of NHPs needed in future experiments.
- Development of a rabbit model for passive immunization studies against *Bacillus anthracis* results in a reduction in the number of NHPs used for future studies.
- Using cell culture assay for initial determination of antiviral activity greatly reduces (>90%) the number of compounds requiring testing in animals.
- Isolated perfused porcine skin flap technique generates more precise data, thus reducing the number of experimental replicates (animals) needed to obtain data on the dermal absorption of chemicals.
- Combining teaching labs and other labs that use the same animals decreases the number of animals used by 50%.
- A technique to record *in vivo* electrophysiological activity of brain neurons by implanted telemeters allows more data to be obtained from one animal over long time periods instead of using many animals for short periods.

- Development of non-animal systems such as cell culture for virus titrations and plaque-reduction neutralization tests in Encephalomyelitis virus (VEE, EEE, and WEE) challenge studies has substantially reduced the use of animals for these determinations.

- Cell culture studies will be used to characterize infectivity of preparations before animals are used thereby reducing the number of animals needed.

Refinement:

- Biomechanical comparison of electrosurgical and scalpel incisions has been refined by the use of state-of-the-art surgical equipment and technique combined with long-acting post-op analgesics and specially designed recovery area.

- Development of a preparation of viral antigens in a rabbit kidney cell line with rabbit serum or in serum-free culture should result in less reaction by the animals.

- Propagating the Leishmania organism in the tail of the jird greatly reduces the apparent distress experienced by the subject.

- Development of a training program on the primate equilibrium platform will make it possible to demonstrate subtle toxic effects on performance at doses that do not produce frank toxicity or anything approaching life-threatening condition.

VI.2 DoD Implementation of Animal Use Alternatives

DoD research protocols strive to minimize the number of animals used to accomplish the program mission and goals. During the review of protocols by the Institutional Animal Care and Use Committee (IACUC), investigators are specifically asked to present information indicating that "Reduction, Refinement, and Replacement" have been addressed in the animal study. Implementation of these alternatives reduces, replaces and refines the Department's use of animals in research. This is accomplished by the implementation of both general and specific alternatives. General alternatives are those that are frequently implemented in many different DoD programs. Specific alternatives are those that may be specific to both a research protocol and/or facility. In FY97, over 400 animal use projects reported that they were implementing alternative methods to the use of animals. Since over 600 general and specific alternatives were implemented by the DoD this year, it is impossible to present all of them in this report. The following examples are a representative listing of general alternative methodologies commonly practiced in DoD facilities:

Replacement:

- During the review process, all potential methods of adequately answering the research objective are reviewed prior to the use of an animal model.

- The evaluation process also considers the selection of a particular animal type; species lower on the phylogenetic scale are considered and used if its selection permits attainment of the research objectives.

- Non-animal training aids are used to reduce the number of live animals required.

Reduction:

- All animal use protocols are subject to review by a biostatistician who addresses the animal used, study design, and statistical evaluation packages, and ensures that the minimum number of animals will be used to meet the specific scientific objectives.

- Pilot studies are used to refine techniques and define the animal model so that animal use can be kept to the minimum required for statistical significance.

- When possible, protocols make use of a repeated measures design and each animal serves as its own control, thereby reducing the number of animals necessary for a particular study.

- Sharing of animal tissues with other investigators reduces animal use.

- Collaboration between DoD investigators allows for a single animal to be used in multiple training and research procedures and the sharing of control group information resulting in an overall reduction in the number of animals used.

- Several types of data are collected simultaneously.

- Training sessions are designed to use the highest student-to-animal ratio that is practical.

Refinement:

- Parameters indicating moribundity rather than death are used as experimental endpoints when possible.

- Animals are anesthetized during euthanasia to decrease stress. Tissues will be maximally utilized to decrease the number of animals needed to supply tissues for this experiment.

- Moribund animals are humanely euthanized to prevent unnecessary pain or distress.

- Utilizing the environmental enrichment strategy, animals are housed in social settings (i.e., pairs or groups) in an enriched environment (e.g., nestboxes, toys).

Specific alternatives implemented by the DoD in FY97 were categorized as a subset of replacement, reduction or refinement and are shown in Table VI-1. These categories illustrate the broad-based spectrum of alternatives to be implemented by the DoD. A representative listing of the specific alternatives is presented in Appendix P.

In addition to the implementation of alternatives, the DoD has established policies specific to the refinement of animal use. For example, Walter Reed Army Institute of Research (WRAIR) has established a policy that mandates consideration of environmental enrichment for research animals. This policy allows for flexibility and creativity for improving conditions of laboratory animals.

***Table VI-1** Alternatives Categories*

Replacement:
• Non-mammalian species or species lower in the phylogenetic scale
• Biochemical/physical methods
• Computer simulations
• Other species replace companion animals
• Replacement using *in vitro* cell cultures
Reduction:
• Utilization of alternative biological testing method
• Substitution of computer simulations or other technologies
• Substitution of another species
• Changes in endpoint measurements
Refinement:
• Reduce pain
• Reduce distress
• Research models and animal alternatives
• Environmental enrichment and improved animal handling

VI.3 DoD Initiatives to Promote Animal Alternatives

The DoD has established a variety of initiatives and targeted programs that are currently in place to promote alternative methods that will refine, reduce and replace the use of animals. These programs are designed to target individual and institutional awareness by providing educational opportunities, professional training, and fiscal resources toward implementing the *Four Rs* approach to animal use.

VI.3.1 Science and Technology Objectives to Reduce Reliance on Animal Research

The Department of Defense continues to seek alternatives to animal use through an Army Science and Technology Plan (STEP) initiated in FY93 and continuing through FY04 titled *Reducing Reliance on Human and Animal Subjects of Research and Improving Experimental Conditions Using Animals.* The objectives of the STEP is to conduct basic research to develop new technologies to incrementally reduce future reliance on research animals. The U.S. Army Medical Research and Materiel Command

(USAMRMC) Medical Biological Defense Research Program budgeted approximately $558,000 in FY97 for this objective, which is available to support alternatives to animal use research.

VI.3.2 DoD-Sponsored Conferences and Workshops on Alternatives to Animal Use

The DoD promotes responsibility for alternatives to animal use by sponsoring major meetings and conferences on the subject. Every 2 years the DoD sponsors an international meeting at Aberdeen Proving Ground on Alternatives to Animal Testing (Table VI-2).

The 1992 meeting had 35 scientific platform sessions and 22 scientific poster presentations. This international symposium was attended by nearly 300 military and civilian scientists from four countries. Proceedings of the 1992 symposium are available through the Defense Technical Information Center (DTIC). In addition, in 1994 a book edited by Dr. Harry Salem titled "Animal Test Alternatives" was published by Marcel Dekker, Inc., which included chapters prepared by most of the presenters at this symposium. The 1994 meeting had 26 scientific platform sessions, including one by Dr. Martin Stephens of the Humane Society of the United States, and 45 scientific poster presentations. This meeting was attended by over 330 military and civilian scientists from seven countries. The proceedings and a monograph based on this successful symposium are available through DTIC. The book "Advances in Animal Alternatives for Safety and Efficacy Testing" has been published by Taylor and Francis. The 1996 conference was coordinated with the Scientists Center for Animal Welfare which held its meeting 10-11 June 1996 to present Animal Welfare and Toxicology/Safety Studies: Current Issues and Trends for the Next Century. The DoD will sponsor another symposium on alternatives to animal use in 1998.

***Table VI-2** DoD-Sponsored Alternatives*

Date	Title
1990	DoD Initiatives in Alternatives to Animal Testing
1992	Current Concepts and Approaches on Animal Test Alternatives
24-26 May 1994	Alternatives in the Assessment of Toxicity: Theory and Practice
12-14 June 1996	Biennial International Symposium on Alternatives in the Assessment of Toxicity Issues, Progress and Opportunities

VI.3.3 National Research Council, Institute of Laboratory Animal Research, Educational Programs

The DoD's priority and continuing commitment to promoting individual and institutional responsibility for alternatives to animal use are reflected in continuing financial support of the Institute of Laboratory Animal Research (ILAR) educational program of the National Research Council. The principal thrust of the ILAR grant is development of institutional training materials, educational courses, and publications in support of the Department's laboratory animal care and use programs. This ILAR information is used in various military research facilities as an important adjunct to existing investigator training and technical education programs on animal care and use. The ILAR information and programs have generated strong animal alternative provisions for both civilian and military-specific research opportunities. The Department has funded this work since 1987 through 5-year grants, and is currently providing funding under the third such grant. In the face of diminishing research funds, the Department has resolved to maintain this important collaboration by providing in excess of $100,000 annually for the ILAR program.

VI.3.4 DoD's Participation in Other Federal Alternatives Programs

The DoD is also represented on the Interagency Regulatory Alternatives Group (IRAG), which planned and presented a "Workshop on Updating Eye Irritation Test Methods" in 1991 and held another workshop on dermal testing held at the American College of Toxicology, in November 1995. The DoD representative on the IRAG (Dr. Harry

Salem) received the Food and Drug Administration's (FDA's) Group Recognition Award for his outstanding contributions to the IRAG (Appendix Q).

The National Institutes of Health Revitalization Act of 1993 (Public Law No. 103-43, Section 1301) directed the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH) to establish an Applied Toxicological Research and Testing Program which represents the NIEHS' component of the National Toxicology Program. The Act further directed the NIEHS to "(a) establish criteria for the validation and regulatory acceptance of alternative testing methods, and (b) recommend a process through which scientifically validated alternative methods can be accepted for regulatory use." To fulfill this mandate, an ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods (ICC-VAM) (the Committee) was established in 1994 by NIEHS to develop a report recommending criteria and processes for validation and regulatory acceptance of toxicological testing methods that would be useful to Federal agencies and the scientific community. The Department of Defense participated in this effort that resulted in a report on the validation and regulatory acceptance of toxicological test methods.

Presentations have also been made on alternatives to the Board of Scientific Councilors of the National Toxicology Program of the National Institute of Environmental Health Sciences (NTP-NIEHS), Board of Scientific Councilors of the Food and Drug Administration and Cancer Etiology Group at the National Cancer Institute.

VI.3.5 Institutional Animal Care and Use Committee Emphasis

Title 9 (Animals and Animal Products), Subchapter A (Animal Welfare), Parts 1-4 of the Code of Federal Regulations has specific provisions for addressing the issue of alternatives during the research animal protocol review process. The DoD has been a leader in forming lawfully constituted and functioning IACUCs at its biomedical research facilities. Accordingly, DoD IACUCs consider alternatives to the proposed use of animals as an important review consideration. All DoD programs use a Standardized IACUC Protocol Format for animal use proposals, which requires that non-animal alternatives be considered. It states that "No study using animals should be considered prior to the elimination of all reasonable possibilities that the question might be adequately answered using other than animal means." Investigators must provide information on the animal model being proposed and justification for the selected species. The Standard Protocol Format states that "investigators should use the least sentient species that will permit the attainment of research objectives." In addition, the investigators are required to provide a short description of the features of the proposal that may qualify the study as one that refines, reduces or replaces the use of animals. The DoD 1995 Policy Letter requires that extramural contractor proposals utilizing animals in research, testing or training include all the information contained in the DoD Standard Protocol Format, thereby requiring them to also provide the alternatives information.

VI.3.6 Veterinary Staff Expertise and Assistance Visits

The major biomedical research commands of the Military Departments each have credentialed laboratory animal medicine (LAM) veterinarians serving in key staff positions. More than 30 board-certified specialists of the American College of Laboratory Animal Medicine (ACLAM) currently serve in the DoD. In addition to being advisors to commanders on issues related to animal welfare and alternatives to animal use, these veterinarians provide oversight and structure to the command's animal care and use programs. These officers also make periodic staff assistance visits to subordinate facilities that use animals and evaluate each laboratory animal care and use program. Consideration of the use of alternatives is reviewed on these staff assistance visits. Another important responsibility of the LAM veterinarian is to review extramural animal use protocols, ensuring that alternatives to animal use and personnel training issues have been addressed.

VI.3.7 Professional Veterinary Training in LAM

The individuals who are specialty trained in veterinary LAM provide expertise in DoD biomedical research institutions, which strongly

correlates to effective animal use alternatives programs. This is especially true in the critical area of refinements. The DoD has long been a leader in training veterinarians in the field of LAM, the biomedical and veterinary specialty most closely associated with laboratory animal welfare and laboratory animal care and use programs. Many of the nationally prominent leaders of several laboratory animal associations were formally trained in, or closely associated with, DoD LAM training programs. Examples are the President and several past presidents of ACLAM, the President-elect and several past presidents of the American Association of Laboratory Animal Science (AALAS), and several past presidents and the current Secretary-Treasurer of the American Society of Laboratory Animal Practitioners. This traditional DoD strength in LAM expertise strongly enhances both animal care and use and animal alternatives programs. Greater than 25% of all ACLAM boarded specialists in the U.S. received some or all of their LAM training in DoD LAM training programs.

VI.3.8 AALAS Technician and Laboratory Animal Science Training

There are a number of DoD research facilities that sponsor formal training programs leading to certification of animal care and research personnel as AALAS laboratory animal technicians. This specialized training is offered to both government and non-government animal technicians. It is an important mechanism for ensuring highly qualified animal care and research technicians in Defense laboratories. Individual DoD institutions have sponsored formal seminars for research personnel where experts from the National Agricultural Workshop curriculum include formal training and information on alternatives to animal use. In addition, WRAIR offers quarterly a workshop on ethical and administrative issues relating to animal use. The AALAS technicians' course curriculum and the WRAIR workshop curriculum include formal training and information on alternatives to animal use.

VI.4 Summary

Each year new techniques and capabilities improve the handling, treatment, and use of animals in research and testing, and potentially reduce the need for animals in those same endeavors. In FY97, there was ample evidence of the DoD's aggressive pursuit to develop alternatives to replace, reduce, and refine the use of animals, for example, the developed alternatives highlighted in Section VI.1 and the increase in the number of facilities reporting developing animal use alternatives. In addition to these developmental efforts, animal use data for FY97 indicate the widespread implementation of validated alternatives. Rats and mice continue to replace nonhuman primates and other mammals higher on the phylogenetic scale in vaccine and drug development efforts. These and other examples of the development and implementation of alternatives have translated into reductions in the overall use of animals higher on the phylogenetic scale (see Section V). Animal use alternatives including refinement, reduction, and replacement constitute key initiatives in the biomedical research, testing, education, and training programs of the Department of Defense. The number of large animals used by the military departments over the past decade has been significantly reduced, and some large species are rarely used at all. Dogs, cats, nonhuman primates, and marine mammals collectively represent less than 0.7% of the total animals used in research by the DoD.

SECTION VII
GLOSSARY

Adjuvant: An agent mixed in a vaccine to enhance the immunological protection afforded.

Alternatives to Animal Use: For purposes of this assessment, "alternatives" are defined as encompassing any subjects, protocols, or technologies that replace the use of laboratory animals altogether; reduce the number of animals required; or refine existing procedures or techniques so as to minimize the level of stress endured by the animal. These technologies involve the continued, but modified, use of animals; use of living systems; use of chemical and physical systems; and use of computers.

Analgesic: An agent that relieves pain without causing loss of consciousness.

Anesthetic: An agent that causes loss of the sensation of pain. Anesthetics may be classified as topical, local, or general.

Animal: For purposes of this assessment excluding embryos, animal is defined as any nonhuman member of five classes of vertebrates: mammals, birds, reptiles, amphibians, and fish. Within this group, two kinds of animals can be distinguished, warm-blooded animals (mammals and birds) and cold-blooded animals (reptiles, amphibians, and fish). Under this definition, invertebrates are not included.

Animal Use: The use of animals for research purposes. Three aspects of animal use are addressed in this assessment: behavioral and biomedical research; testing products for toxicity; and education of students at all levels. This assessment does not cover animal use for food and fiber; animal use to obtain biological products; or animal use for sport, entertainment, or companionship.

Animal Welfare Act: This act, passed in 1966 and amended in 1970, 1976, and 1985, was originally an endeavor to stop traffic in stolen animals that were being shipped across state lines and sold to research laboratories. Amendments to the act have expanded its scope to include housing, feeding, transportation, and other aspects of animal care; however, the act bars regulation of the conduct of research and testing by USDA.

Antibody: Proactive proteins produced by lymphocytes (type of white blood cell) that can specifically bind foreign substances.

Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC): A voluntary private organization that has provided accreditation for over 600 institutions. AAALAC accreditation is based on the provisions of the NRC *Guide for the Care and Use of Laboratory Animals*, and is recognized by the Public Health Service.

Biological Model: A surrogate or substitute for a process or organ of interest to an investigator. Animals or alternatives can serve as biological models.

Biomedical Research: A branch of research devoted to the understanding of life processes and the application of this knowledge to serve humans and animals. A major user of animals, biomedical research affects human health and the health care industry. It is instrumental in the development of medical products such as drugs and medical devices, and in the development of services such as surgical and diagnostic techniques. Biomedical research covers a broad spectrum of disciplines, such as anatomy, biochemistry, biology, endocrinology, genetics, immunology, nutrition, oncology, and toxicology.

Blast Overpressure: The concussion that results when weapons such as artillery pieces are fired. Soldiers firing these weapons can be severely injured by the local pressure effects resulting from weapon use. Blast overpressure occurs when soldiers are fired upon also, i.e., the shock wave from enemy weapon fire/blast.

Carcinogen: An agent or process that significantly increases the incidence of abnormal, invasive, or uncontrolled cell growth in a population. Carcinogens fall into three classes: chemicals, viruses, and ionizing radiation. A variety of screening assays have been developed to detect chemical carcinogens, including the *Salmonella*-mediated mutagenesis assay (Ames test), the sister chromatid exchange assay, and traditional laboratory animal toxicity tests.

Carcinogenesis: The process by which a change to a cell occurs that leads to cancer.

Cell Culture: Growth in the laboratory of cells isolated from multicellular organisms. Each culture is usually of one type. Cell culture may provide a promising alternative to animal experimentation, for example, in the testing of mutagenicity, and may also become a useful adjunct in repeated-dose toxicity testing.

Computer Simulations: The use of specially devised computer programs to simulate cells, tissues, fluids, organs, and organ systems for research purposes: to develop mathematical models and algorithms for use in toxicity testing, and to simulate experiments traditionally done with animals for educational purposes.

Distress: Usually the production of pain, anxiety, or fear. However, distress can also occur in the absence of pain. For example, an animal struggling in a restraint device may be free from pain, but may be in distress. Distress can be eased with tranquilizers.

Education: The aspect of education dealt with in this assessment is the use of animals and alternatives in the teaching of life sciences to health professionals and preprofessionals, and research scientists.

Ex vivo: Outside the living body: denoting removal of an organ, tissue or cells.

Guidelines for Animal Care and Use: Various organizations outside the federal government have adopted their own guidelines -- e.g., the American Psychological Association's *Guidelines for Ethical Conduct in the Care and Use of Animals*, which is comprehensive and has been endorsed by FASEB; the American Physiological Society's *Guiding Principles in the Care and Use of Animals*; and the American Veterinary Medical Association's *Animal Welfare Guiding Principles*. For federal guidelines, see Interagency Research Animal Committee, NRC *Guide for the Care and Use of Laboratory Animals*, and PHS *Policy*.

Institute of Laboratory Animal Research (ILAR): A component of the National Research Council, ILAR performs periodic surveys on the use of laboratory animals.

Institutional Animal Care and Use Committee (IACUC): An institutional committee that reviews research proposals and oversees housing and routine care of animals. The committee's membership generally includes the institution's attending veterinarian, a representative of the institution's administration, users of research animals, and one or more nonscientist and lay member.

Invertebrate: Any nonplant organism without a spinal column, e.g., worms, insects, and crustaceans. Invertebrates account for 90 percent of the earth's nonplant species. For the purposes of this assessment, invertebrates are not considered to be animals.

***In vitro*:** Literally, in glass; pertaining to a biological process or reaction taking place in an artificial environment, usually a laboratory. Human and animal cells, tissues, and organs can be cultured *in vitro*. *In vitro* testing may hold some promising alternatives to animal testing, e.g., in testing for eye irritation and mutagenicity.

***In vivo*:** Literally, in the living; pertaining to a biological process or reaction taking place in a living cell or organism.

Macrophage: A white blood cell that is very active in inflammatory responses and in engulfing foreign objects such as bacteria.

National Research Council's *Guide for the Care and Use of Laboratory Animals*: Revised in 1996, the *Guide* details standards for animal care, maintenance, and housing. It is used by many animal research facilities, both within and outside the federal government. AAALAC and PHS also

use it when assessing research facilities for accreditation.

Organ Culture: The attempt to isolate and maintain animal or human organs in *in vitro* culture. Long-term culture of whole organs is not generally feasible, but they can be sustained in cultures for short periods (hours or days).

Pain: Discomfort resulting from injury or disease. Pain can also be psychosomatic, the product of emotional stress. Pain can be induced by mechanical, thermal, electrical, or chemical stimuli, and it can be relieved by analgesics or anesthetics.

Polymerase Chain Reaction: A molecular biological system in which pieces of genetic material can be synthesized in large amounts *in vitro*. This material can be used in diagnostic testing, genetic studies, or for a large number of molecular biological purposes.

Protocol: The written plan of a scientific experiment or treatment.

Public Health Service *Policy on Humane Care and Use of Laboratory Animals*: Revised in 1985, the Policy applies to PHS-supported activities involving animals (including those of NIH). It relied on the NIH *Guide for the Care and Use of Laboratory Animals* (1985), and uses institutional committees for the assessment of programs and maintenance of records.

Reduction: Considered an alternative to animal use when fewer animals are used in research and education through changed practices, sharing of animals, or better design of experimental protocols.

Refinement: An alternative to animal use by better use and modification of existing procedures so that animals are subject to less pain and distress. Examples of such refinements are the administration of anesthetics and tranquilizers, humane destruction, and the use of noninvasive imaging techniques.

Replacement: An alternative to animal use, replacing methods using animals with those that do not. Examples include the use of a placenta instead of a whole animal for microsurgical training, the use of cell cultures instead of mice and rats, the use of non-living systems, and the use of computer programs.

Research Facility: Under the Animal Welfare Act, any individual, institution, organization, or postsecondary school that uses or intends to use live animals in research, tests, or experiments. Facilities that receive no federal support for experimental work and that either purchase animals only within their own state or that maintain their own breeding colonies are not considered research facilities under the act.

Testing: Standardized procedures that have been demonstrated to predict certain health effects in humans and animals. Testing involves the frequent repetition of well-defined procedures with measurement of standardized biological endpoints. A given test may be used to evaluate many different substances and use many animals. Testing is used to establish the efficacy, safety, and toxicity of substances and procedures.

Tissue Culture: The maintenance *in vitro* of isolated pieces of a living organism. The various cell types are still arranged as they were in the original organism and their differential functions are intact.

Toxicity Testing: The testing of substances for toxicity in order to establish conditions for their safe use. There are now more than 50,000 chemicals on the market and 500 to 1,000 new ones are introduced each year.

Vesicant: A chemical agent that causes burns and tissue destruction both internally and externally.

Veterinary Medicine: The science and art of prevention, cure and/or alleviation of disease and injury in animals. Veterinary medicine includes the management of animal care and use programs.

SECTION VIII
REFERENCES

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